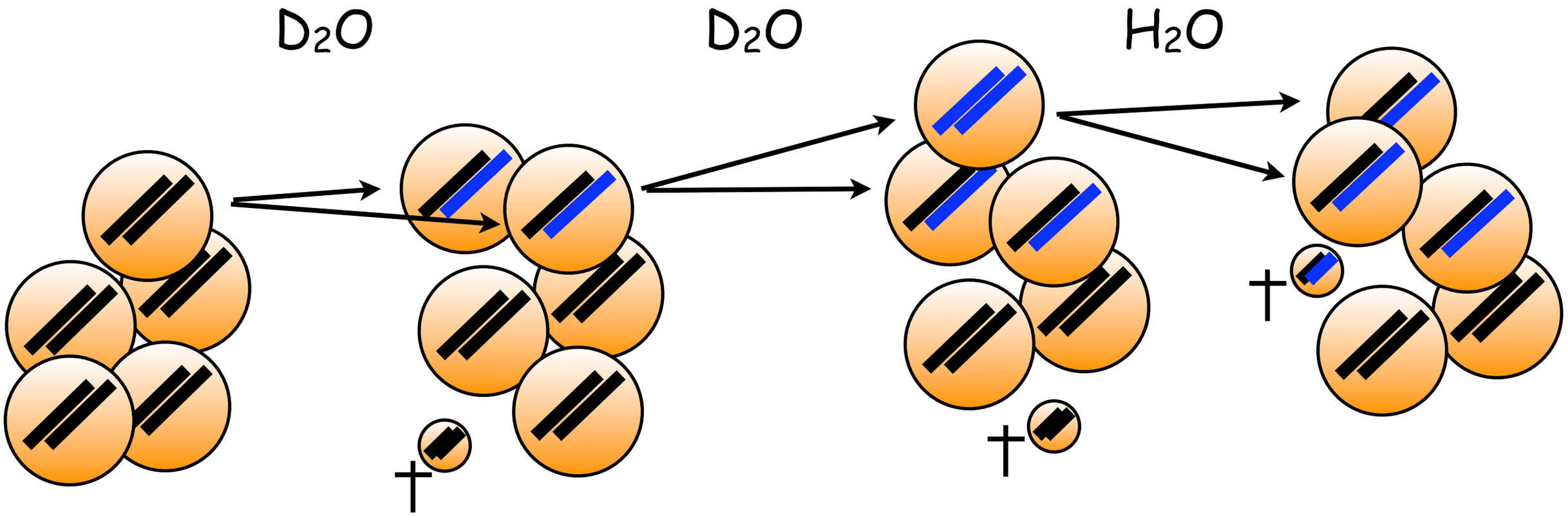
Systems Immunology
(Quantitative Immunology)
requires estimating many parameters.

This can be challenging.

Rob de Boer

Theoretical Biology, Utrecht University, NL

Quantification example 1: Deuterium labeling



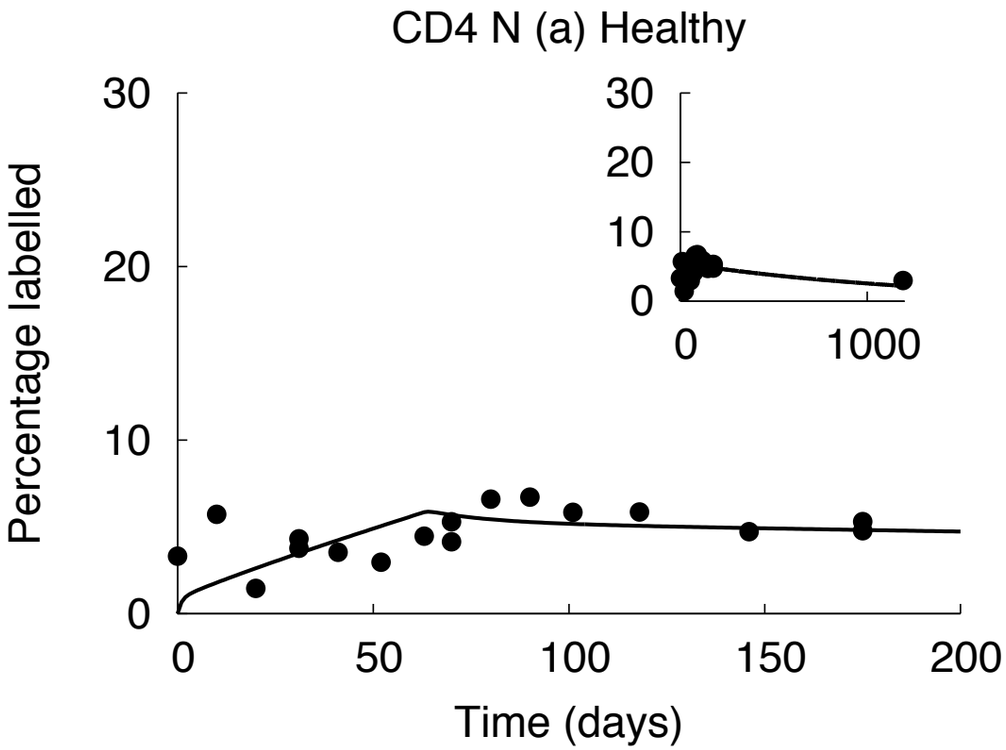
0:10 2:10 4:10 3:10

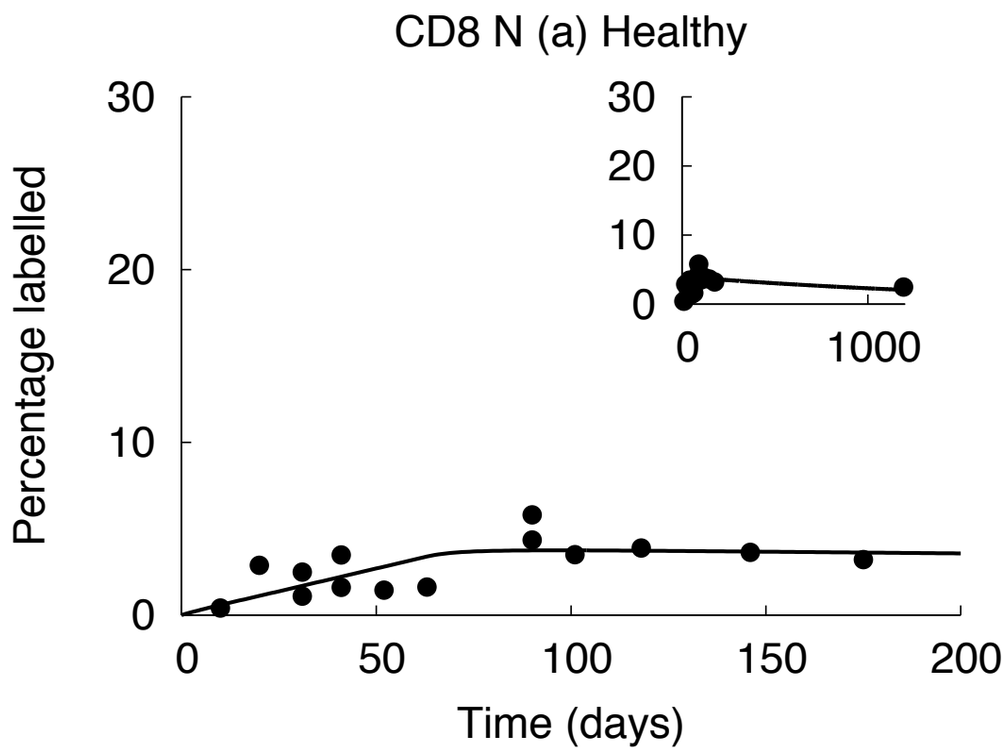
In the presence of deuterium, cell division copies DNA strands into labeled DNA strands: U -> U + L and L -> L + L

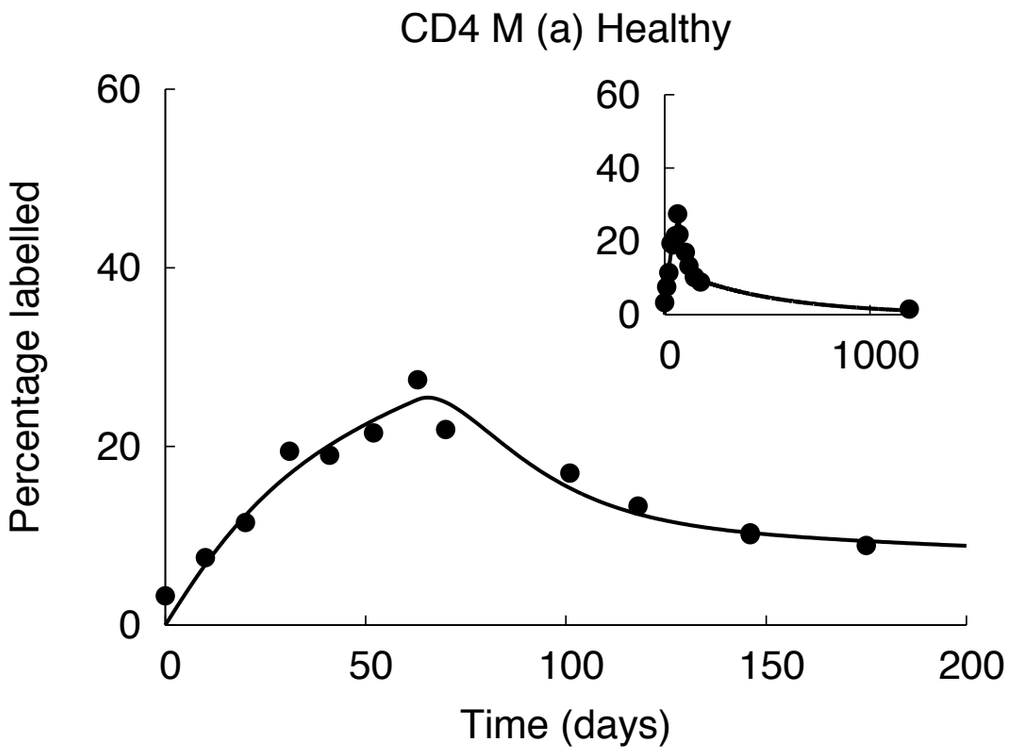
In its absence U -> U + U and L -> L + U

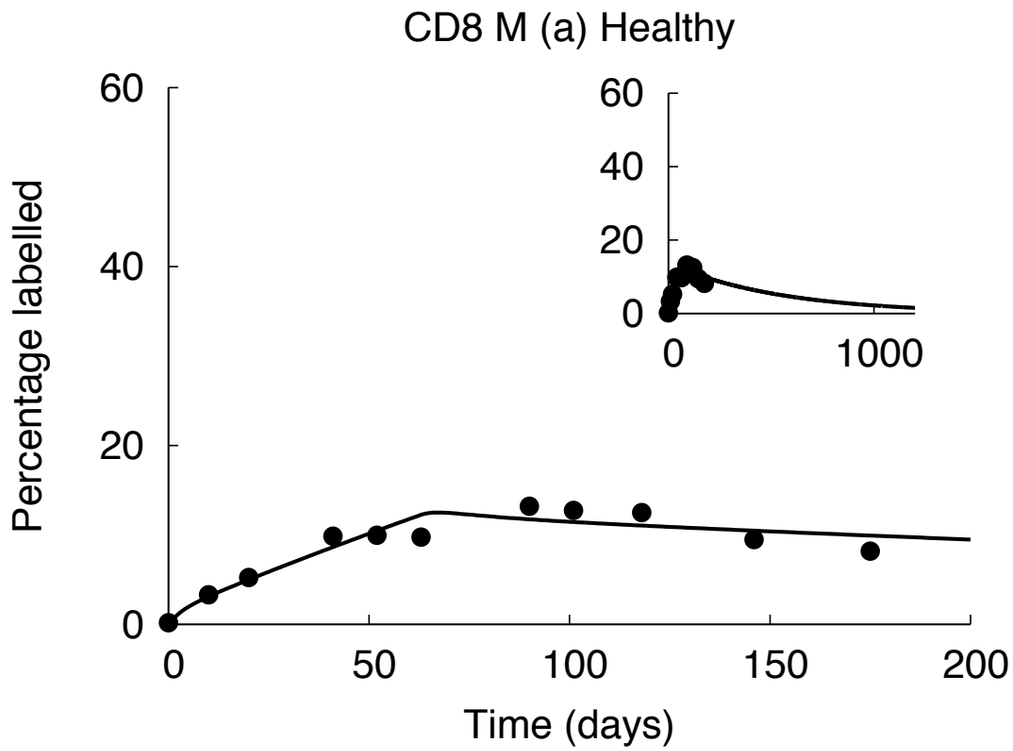
DNA strands can only disappear by cell death

Healthy human volunteers: one individual (a)









Vrisekoop et al PNAS 2008

Results from 5 human volunteers

Expected life spans

Naive $CD4^+$ T cells: 2300 days (6.2 years)

Naive $CD8^+$ T cells: 3300 days (9.1 years)

Memory $CD4^+$ T cells: 160 days (0.45 years)

Memory $CD8^+$ T cells: 120 days (0.33 years)

Compartments:

Fitting the naive T cell data typically requires only one compartment: no evidence for short-lived RTE

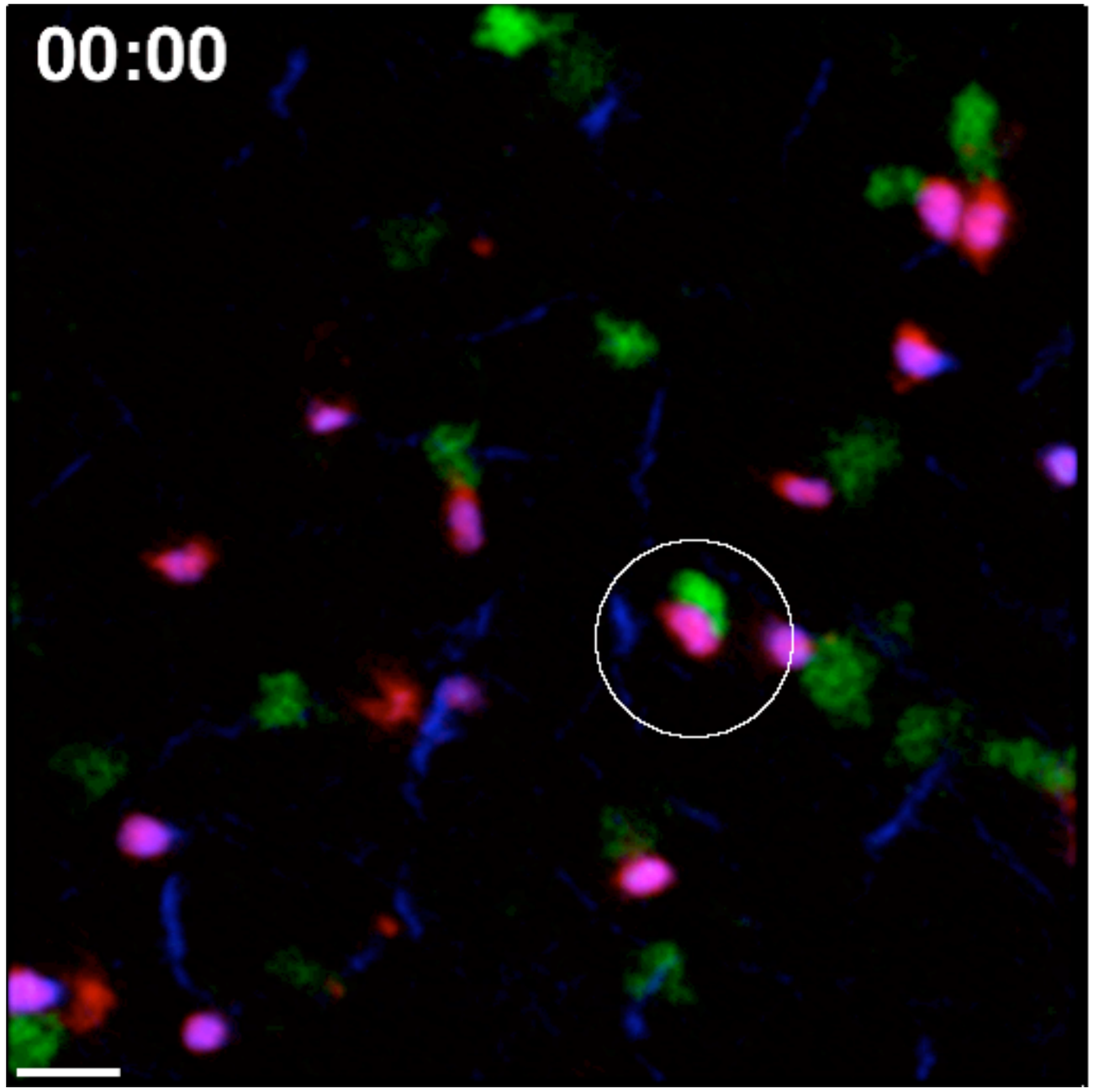
Memory data do require 2 compartments: heterogeneity

Similar results for mice but 50-fold faster

Borghans, Vrisekoop, Den Braber, Mugwagwa, Tesselaar, Miedema

Quantification 2: killing rates of CTL:

2PM movie of Ag pulsed B cells being killed by CTL

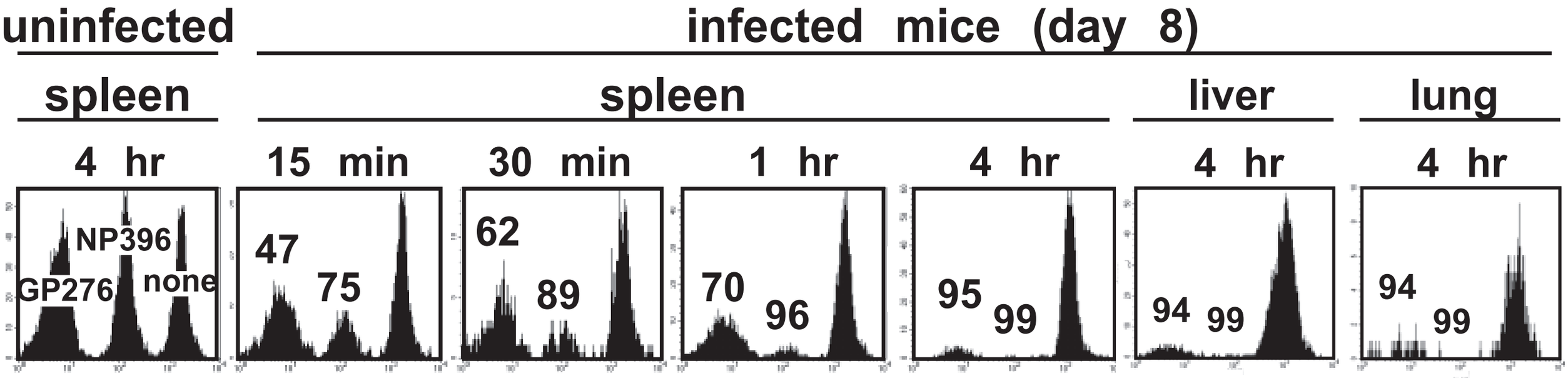


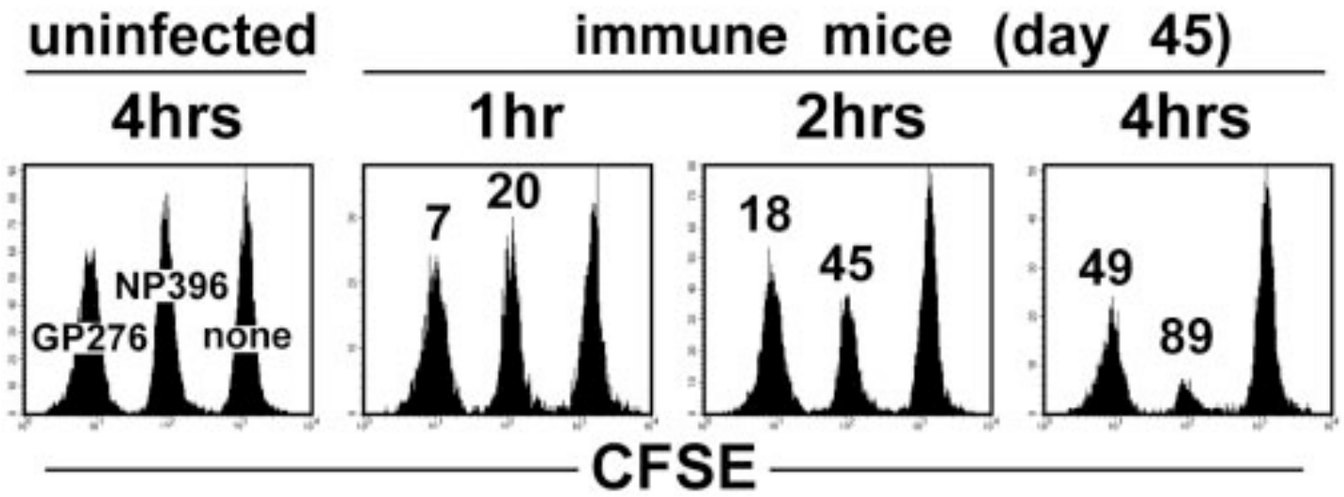
B cell (target cell): purple, CTL: green, death B cell: white

From: Mempel et al. Immunity 2006

Adoptive transfer experiments: Barber et al JI03

- tranfer peptide pulsed splenocytes into mice (GP276 & NP396)
- at peak of LCMV response (d8) or in memory phase (d45)
- compare numbers of pulsed and unpulsed cells in spleen



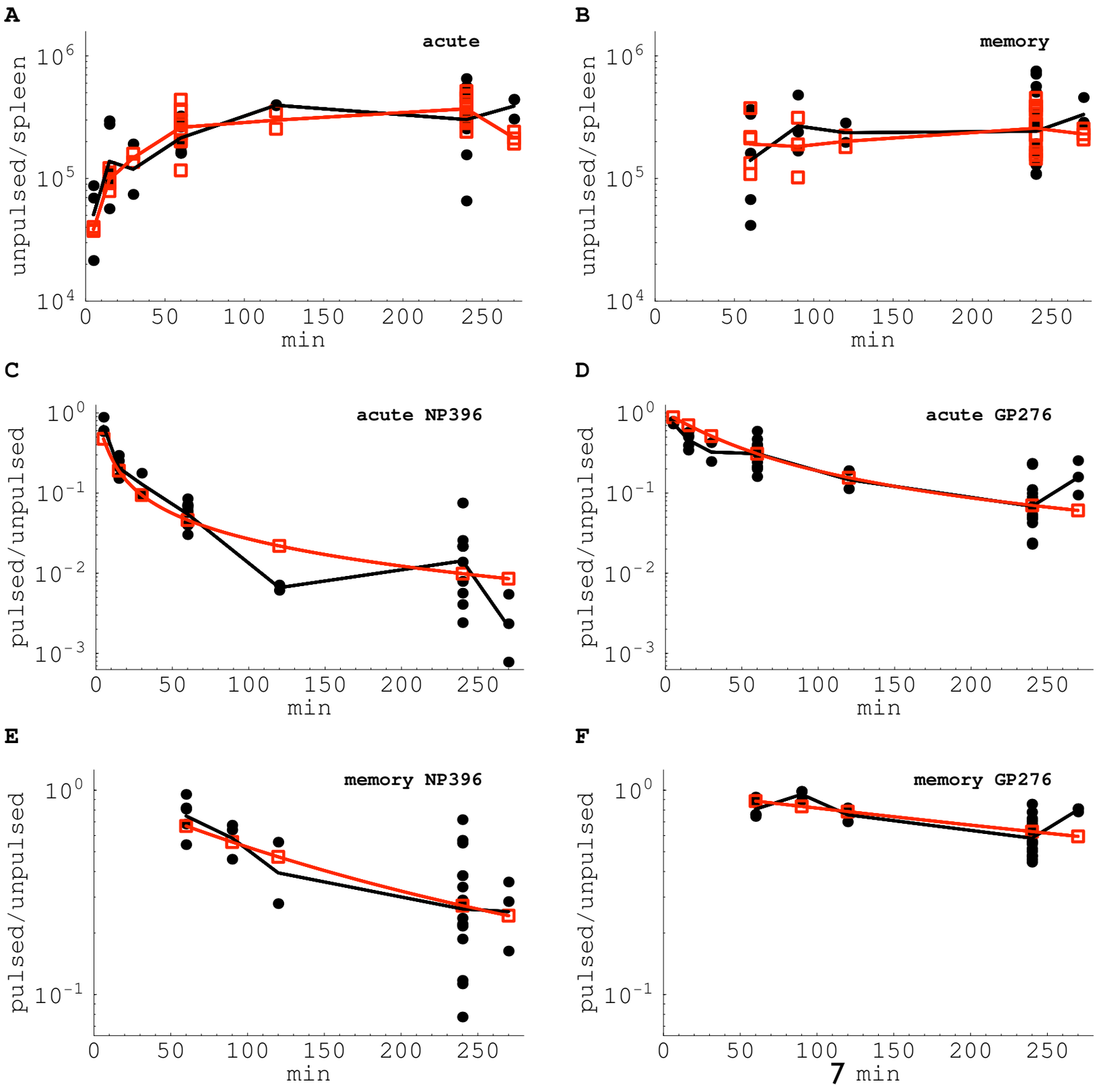


Numbers give percentage target cells killed

high E:T ratio

Very rapid killing of target cells

Modeling the Barber et al data



Antia, Regoes,
Yates, Barber,
Graw, Ganusov,
De Boer

$$T' = \sigma B - (e + K)T$$

Death rates K:

$K^a_{NP} = 497$ d^{-1}

$K^a_{GP} = 72$ d^{-1}

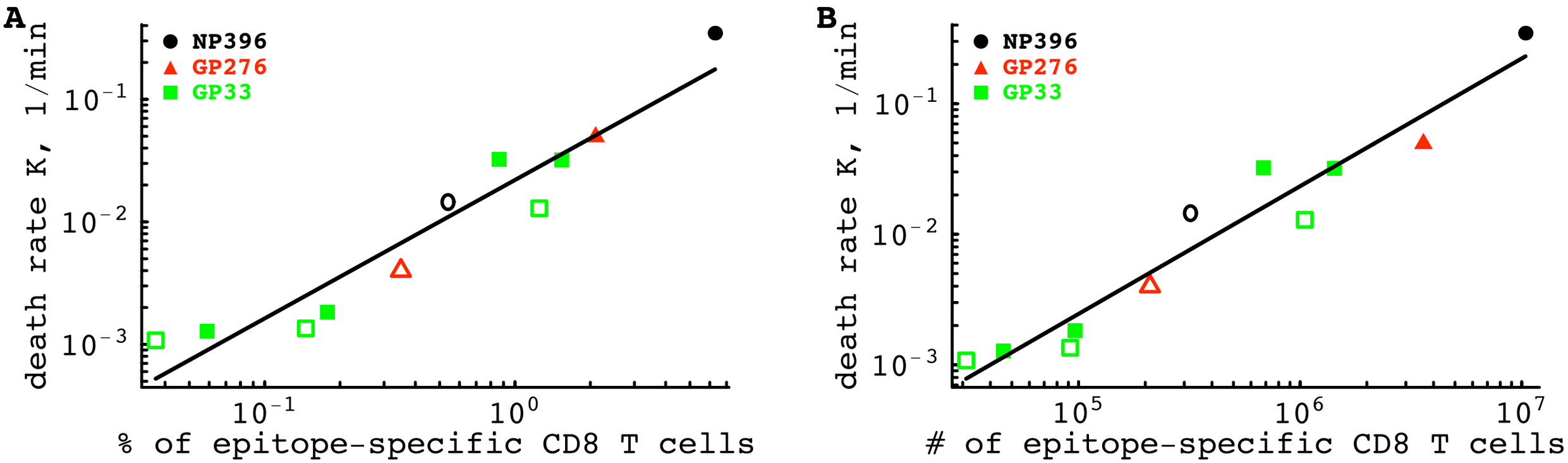
$K^m_{NP} = 21$d $^{-1}$

$K^m_{GP} = 6$ d^{-1}

500 d^{-1} is 3 min

Ganusov & De Boer J Virol 08

Modeling more Barber et al data



Killing seems to follow a mass action term

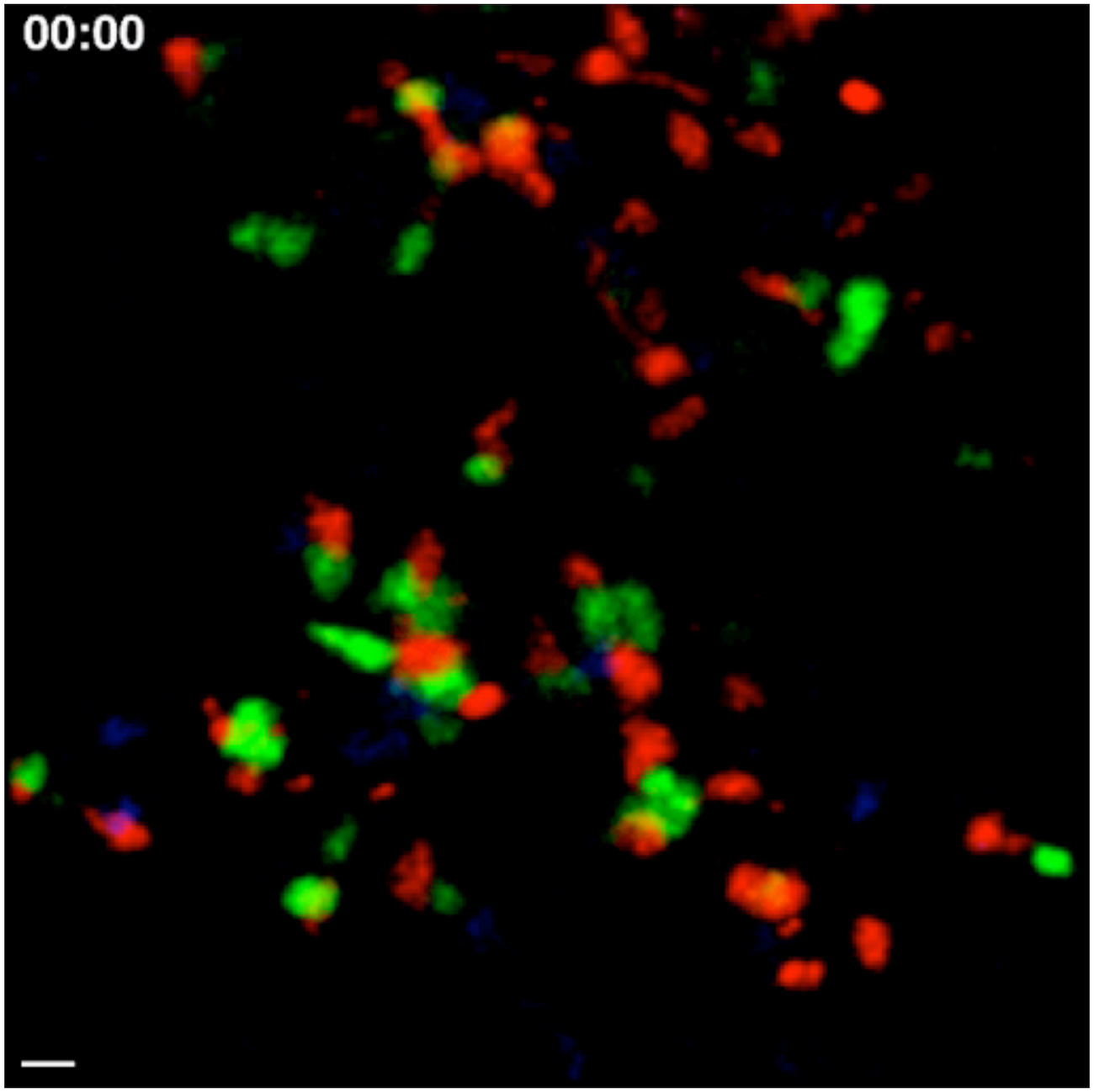
differences between epitopes seems small

One CTL kills KT/E=1-5 target cells per day.

Careful: cells may die during later experiment steps

Ganusov, Barber & De Boer, in prep

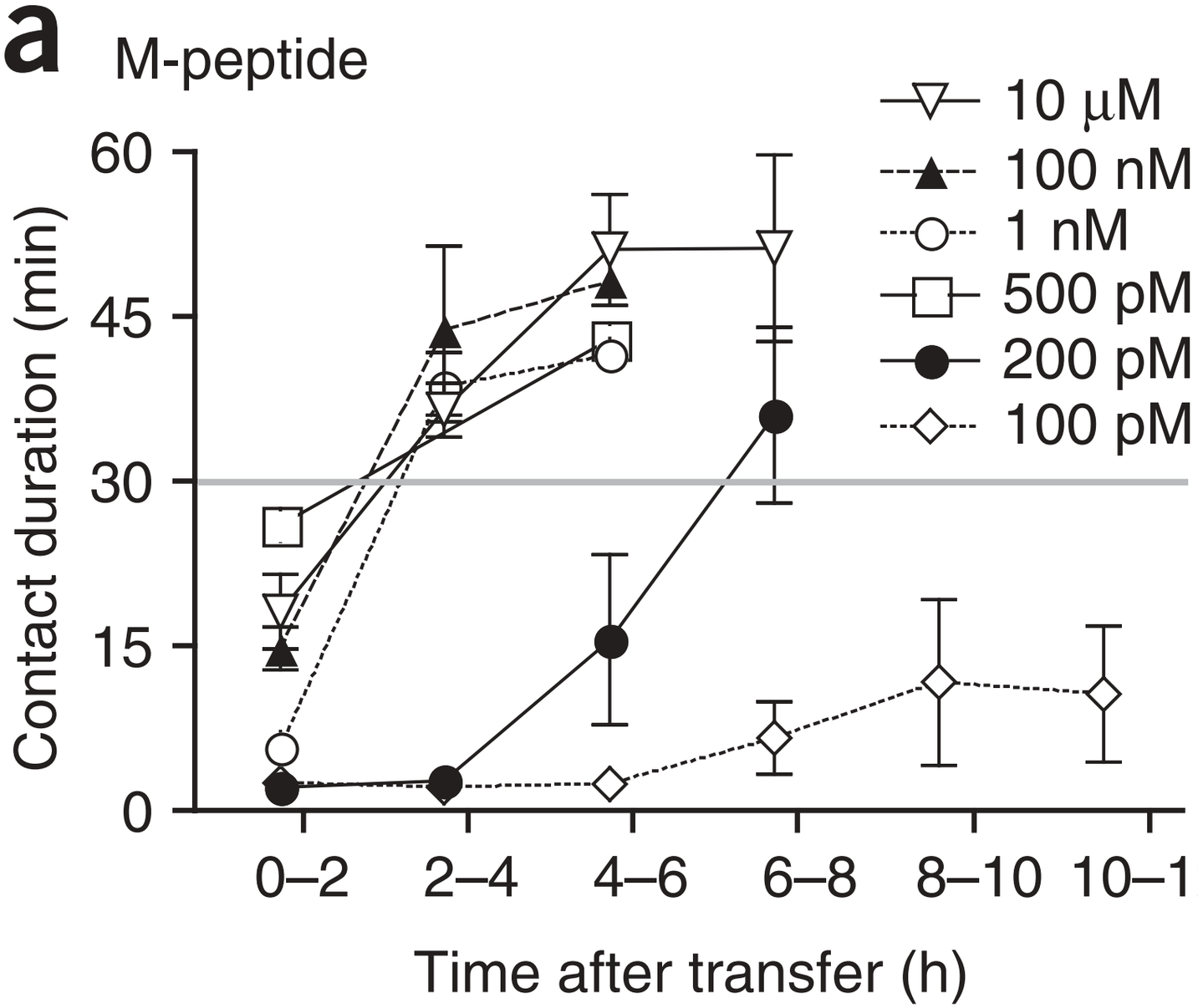
Quantification example 3 (most challenging): Contact times between specific T cells and DC



Green: Ag specific CD8 T cells, Blue control cells, and Red DC

Henrickson et al. Nat. Imm (2008)

Observed contact times increase from phase 1 to 2

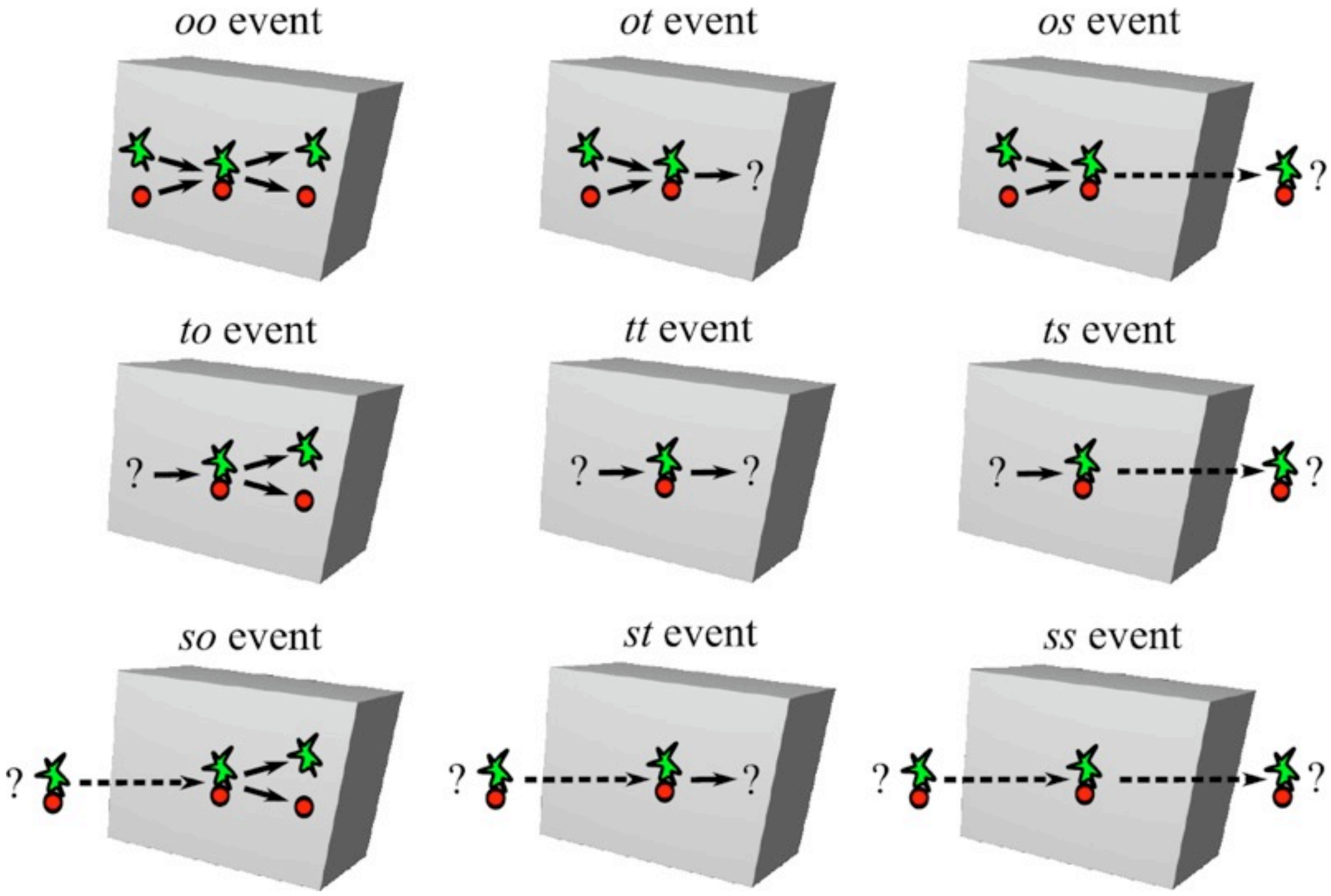


Data from Henrickson et al.
Nat Imm 2004

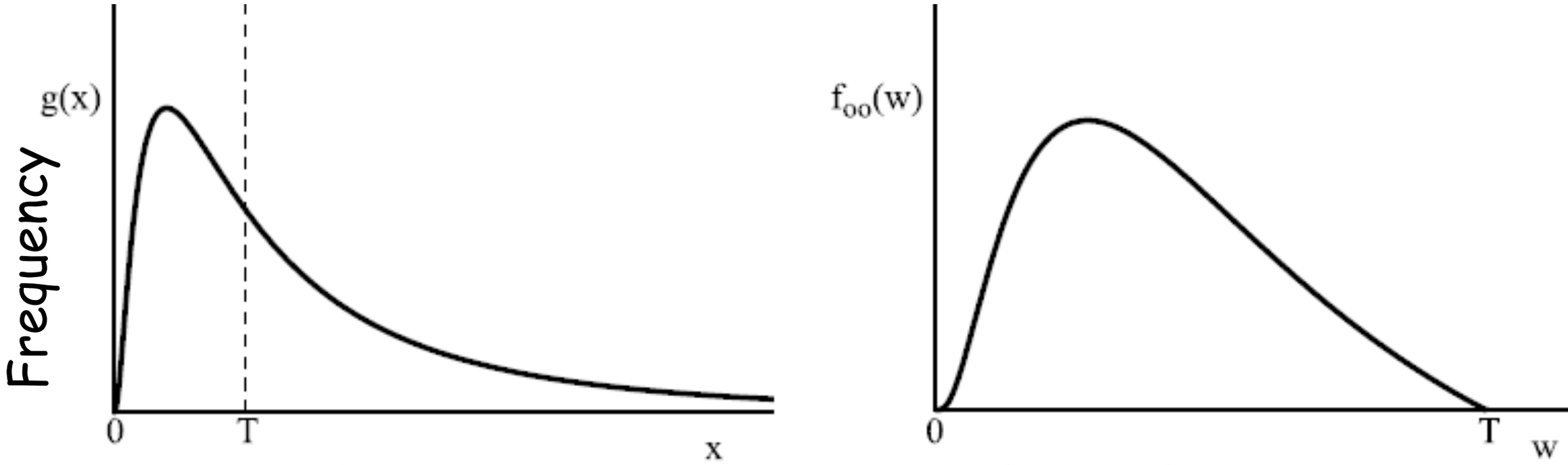
Movies typically last one hour which is shorter than many of the contacts in phase 2:

Difficult to estimate true contact times

From observed to true contact times: complicated problem



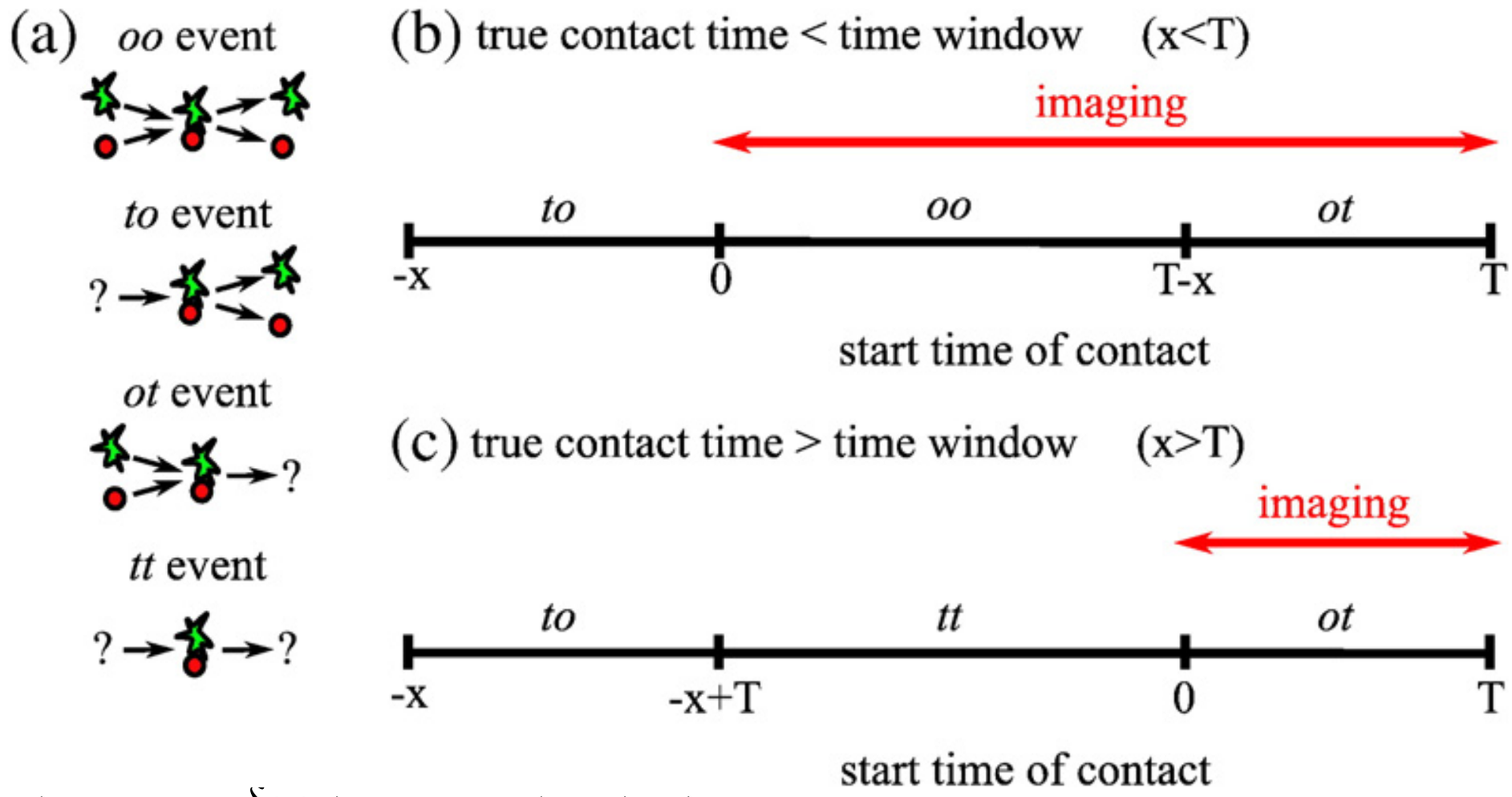
Assume a true contact distribution to predict the observed event distributions



True distribution g(x) gives expected f..(w),
where x is the true and w the observed contact time.
T is imaging time and δ the rate of leaving the area
δ is an average that is not expected to hold for cells that just entered the field

Compute probabilities to observe each event

J.B. Beltman et al. / Journal of Immunological Methods 347 (2009) 54–69



$$f_{oo}(w) = \mathrm{e}^{-\delta w}(T-w)g(w)$$

$$f_{ot}(w) = f_{to}(w) = \mathrm{e}^{-\delta w}\int_{x=w}^{\infty} g(x)\mathrm{d}x \ ,$$

$$f_{tt}(T) = \mathrm{e}^{-\delta T}\int_{x=T}^{\infty}(x-T)g(x)\mathrm{d}x$$

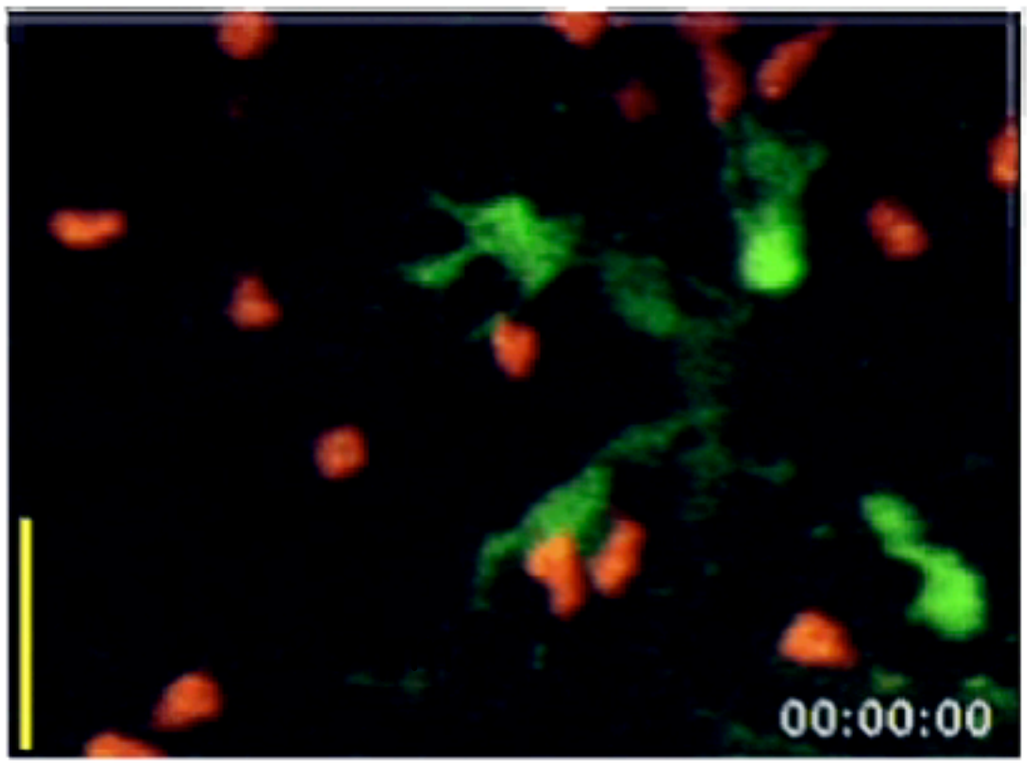
Full model

$$
\begin{aligned}
f_{oo}(w) &= \mathrm{e}^{-\delta w}(T-w)g(w) \\
f_{ot}(w) &= f_{to}(w) = \mathrm{e}^{-\delta w}\int_{x=w}^{\infty} g(x)\mathrm{d}x \\
f_{tt}(T) &= \mathrm{e}^{-\delta T}\int_{x=T}^{\infty}(x-T)g(x)\mathrm{d}x \\
f_{os}(w) &= f_{so}(w) = \delta\mathrm{e}^{-\delta w}(T-w)\int_{x=w}^{\infty} g(x)\mathrm{d}x \\
f_{ts}(w) &= f_{st}(w) = \delta\mathrm{e}^{-\delta w}\int_{x=w}^{\infty}(x-w)g(x)\mathrm{d}x \\
f_{ss}(w) &= \delta^2\mathrm{e}^{-\delta w}(T-w)\int_{x=w}^{\infty}(x-w)g(x)\mathrm{d}x \\
f_{oo}(w)+\ldots+f_{ss}(w) &= \int_{x=0}^{\infty}(T+(1+\delta T)x)g(x)\mathrm{d}x
\end{aligned}
$$

The latter gives the total number of events one expects to observe, normalized to the total number of contacts, N, initiated per hour.

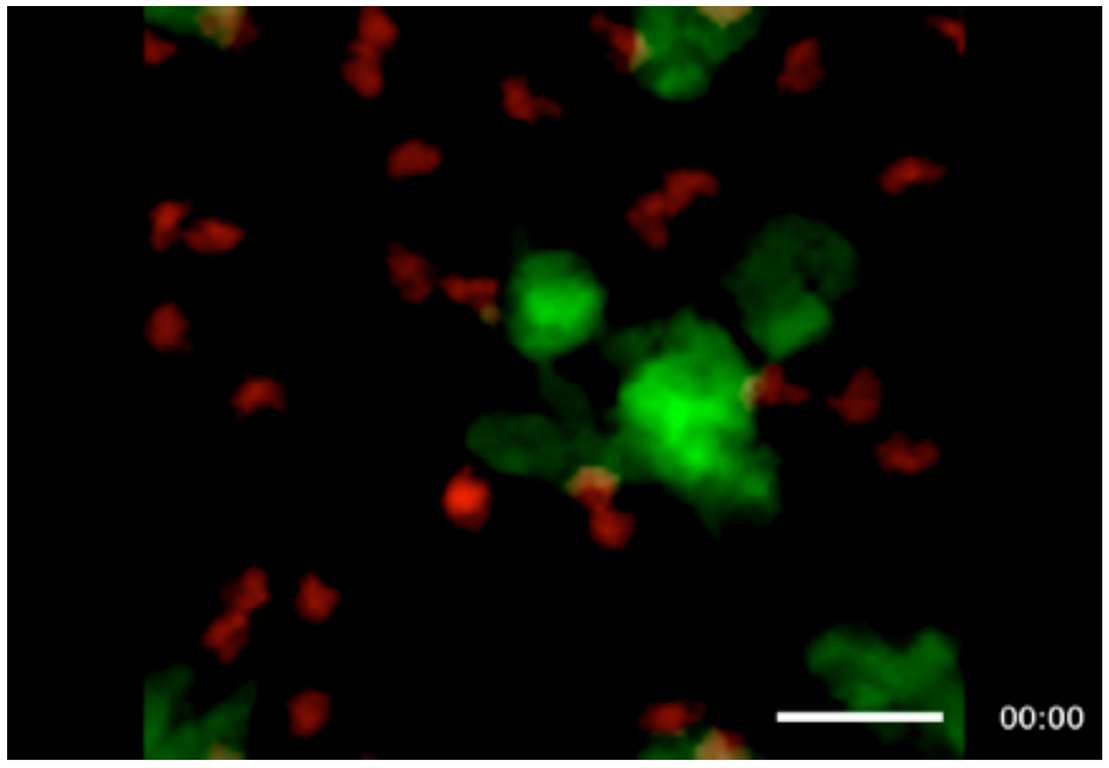
First test the method with our CPM

in vivo



Miller et al. J Exp Med (2004)

in silico (CPM)

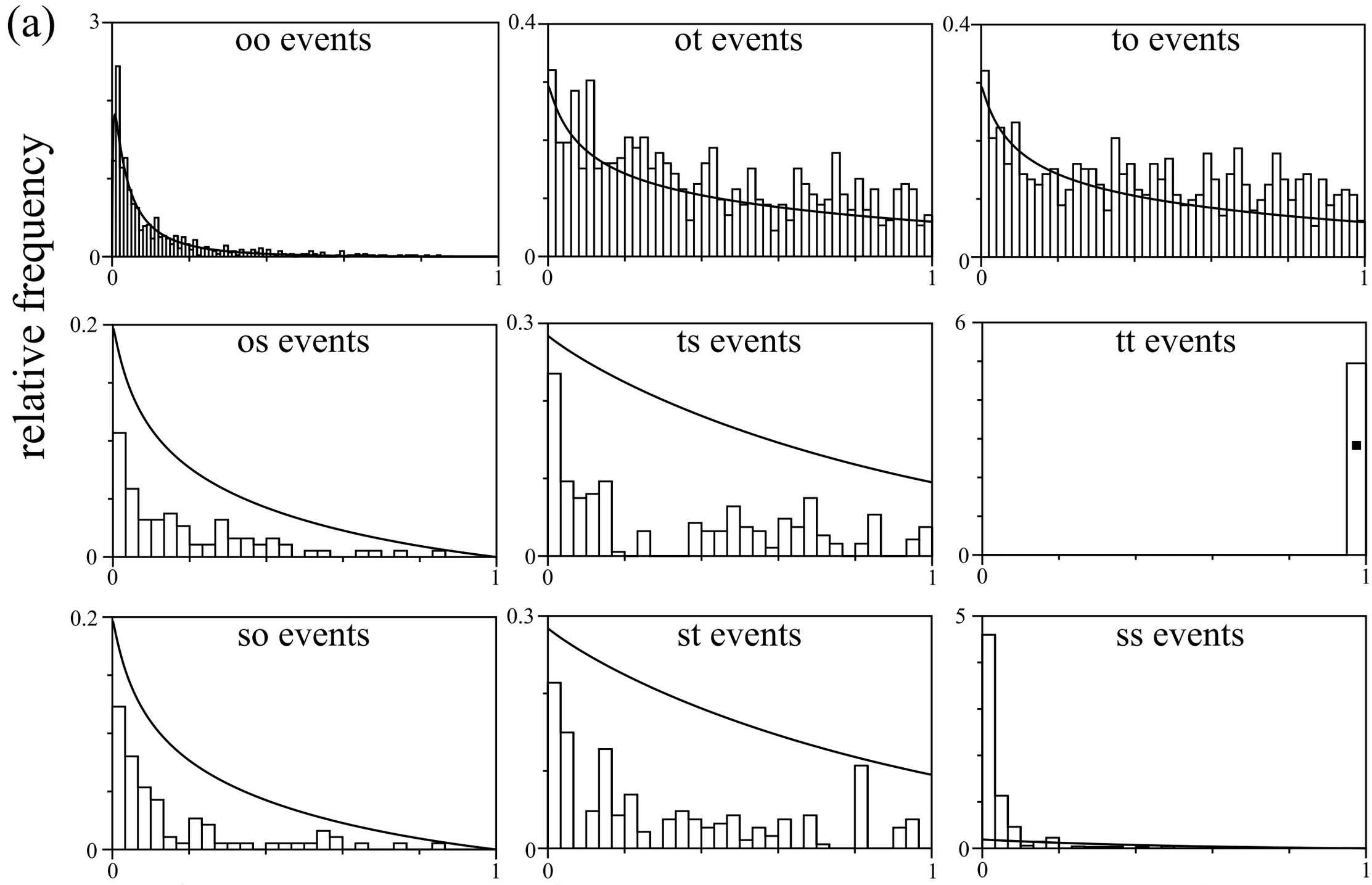


Beltman et al. J Exp Med (2007)

Red: T cells **Green**: Dendritic cells (DC)

Computer model (CPM) with realistic behavior

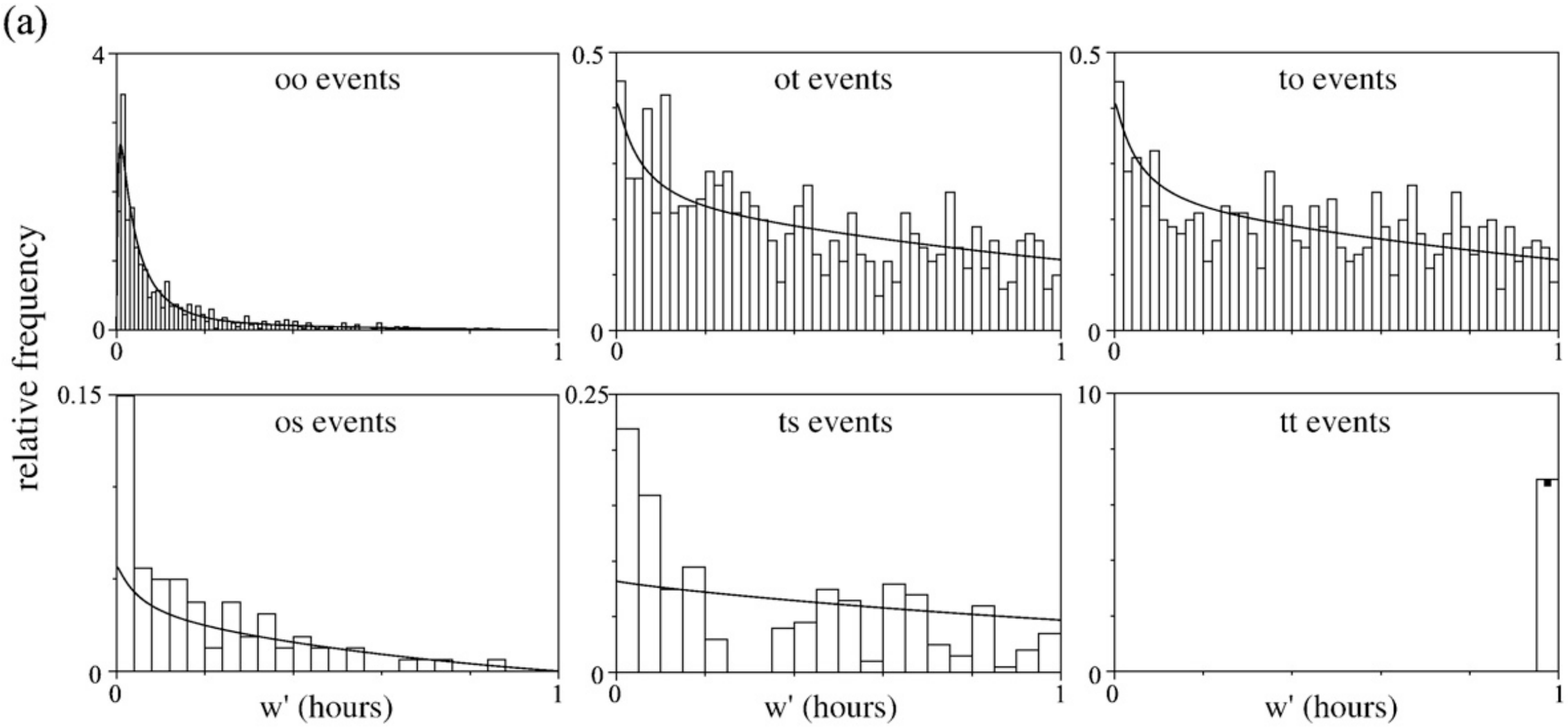
Validate method using CPM simulations



Split 100-h simulations into 100 parts of 1 hour

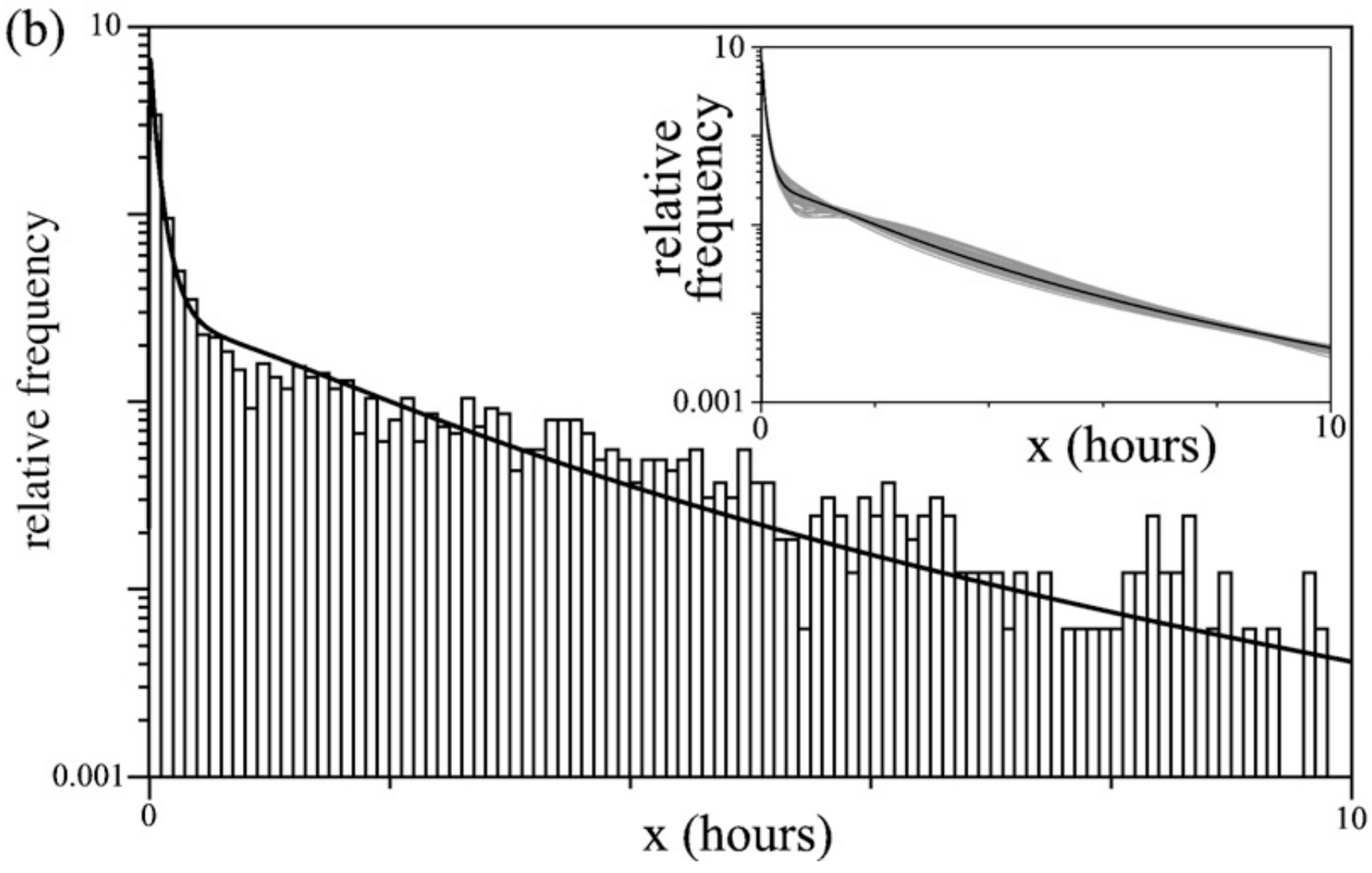
fit observed events by maximum likelihood procedure

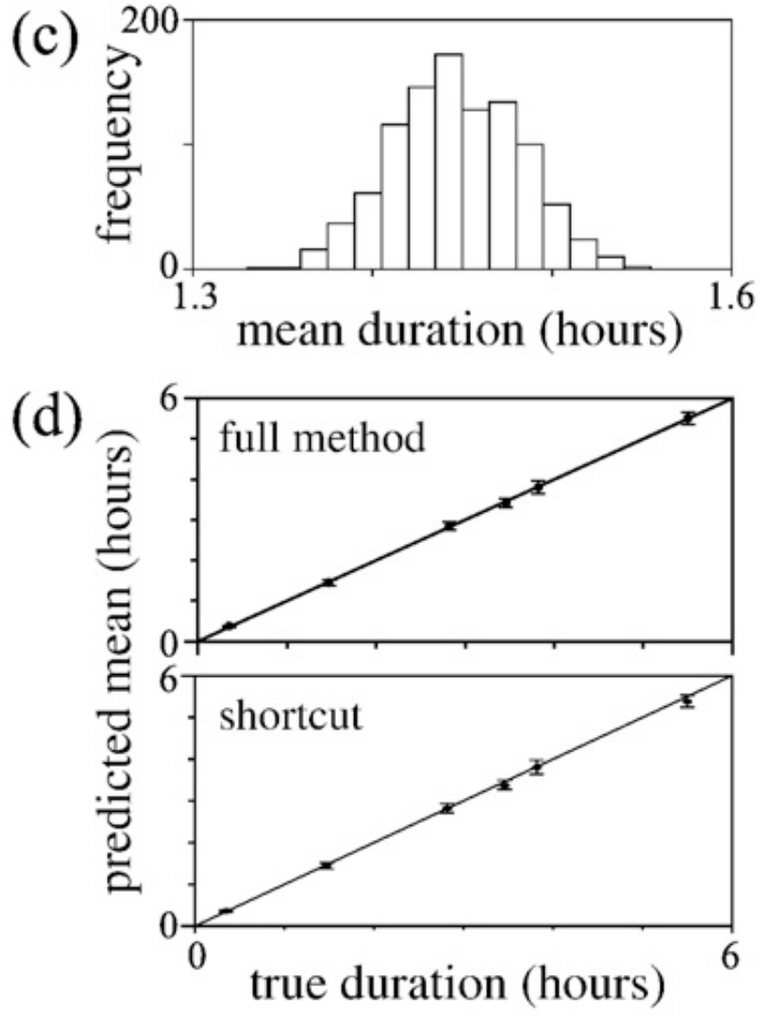
Ignoring all events of entering cells (t.)



gives a much better description of the in silico data

and a correct estimate of the contact times





fitting the sum of two lognormals for g(x)

Beltman et al J Imm Methods 2009

Shortcut method

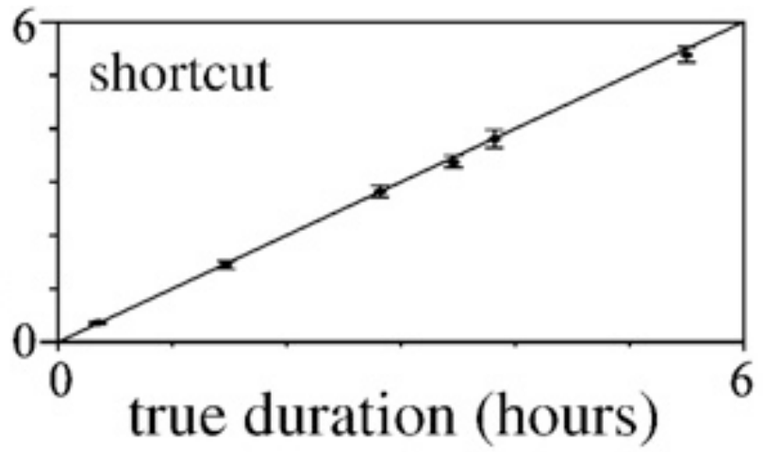
Total number of conjugates at any point in time:

$$\overline{n_C} = N \int_{x=0}^{\infty} x g(x) \mathrm{d}x$$

where N is the number of contacts initiated per hour.

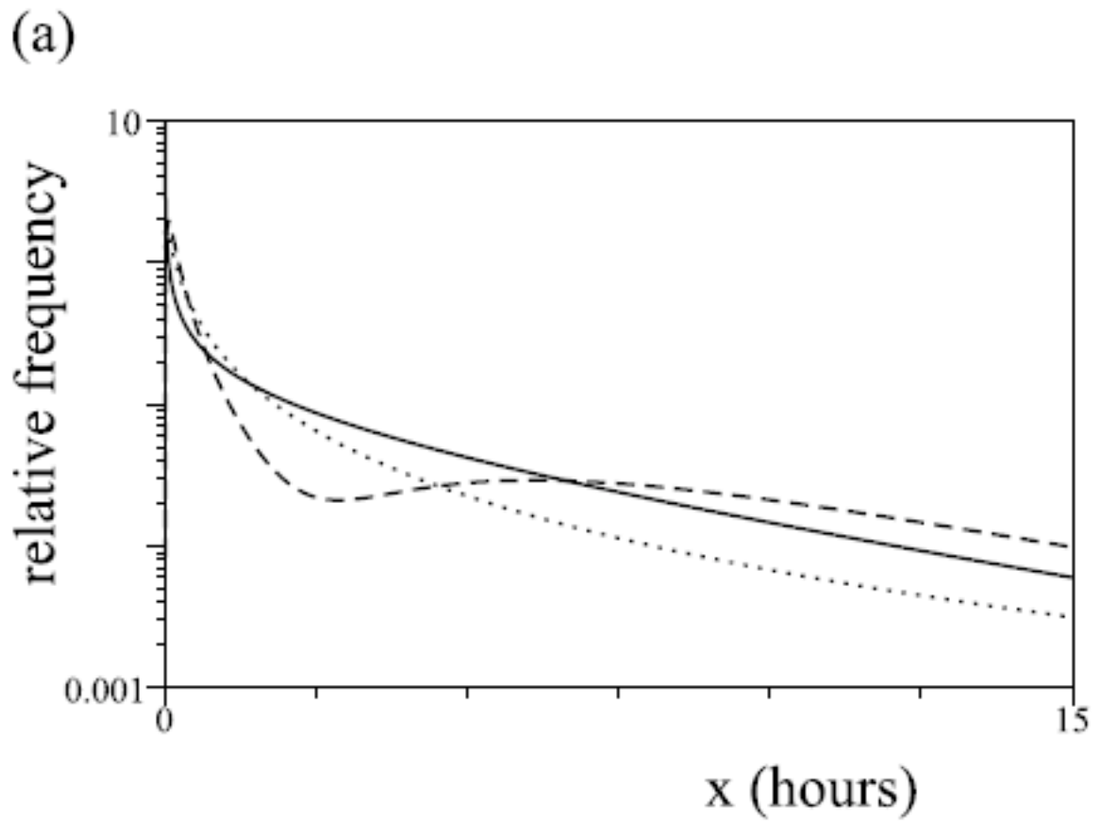
The average contact time can thus be calculated by dividing the (average) number of conjugates by the number of initiation events:

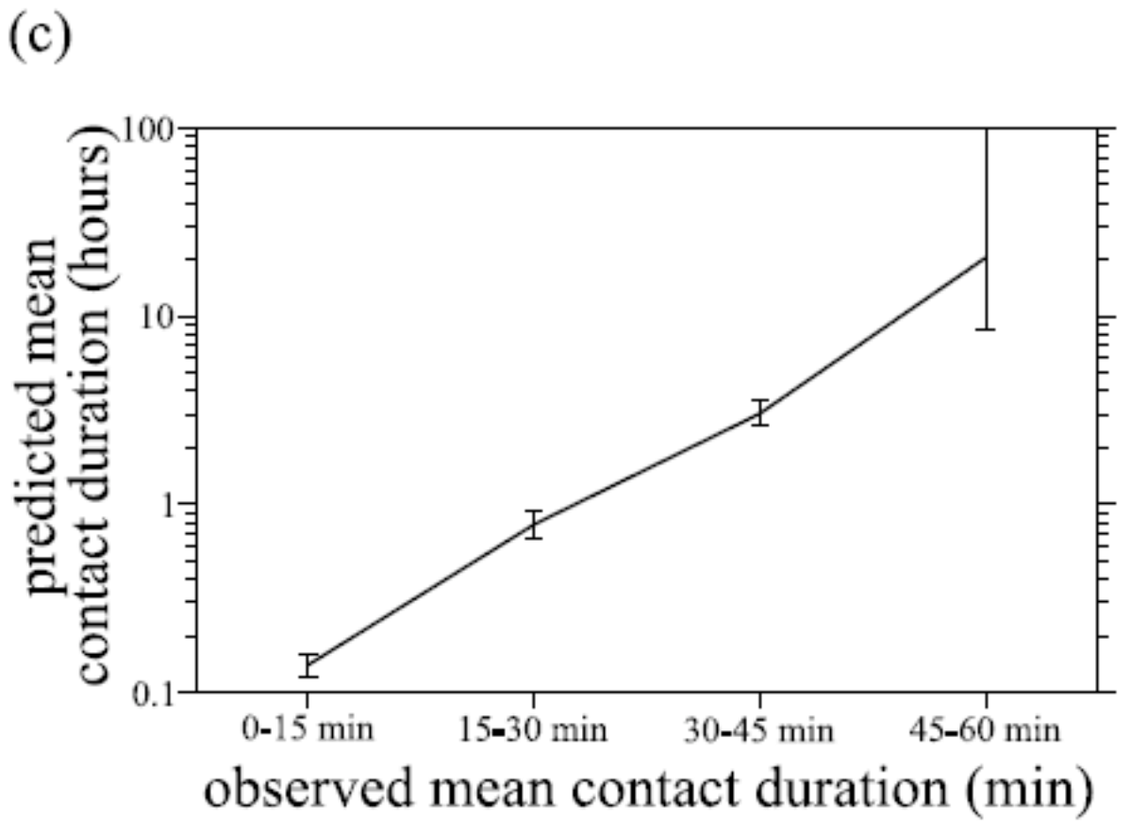
$$\bar{x} = \frac{2T\overline{n_C}}{n_i + n_t}$$



(excluding entries and exits)

Contact data from Henrickson Nat Imm 2008

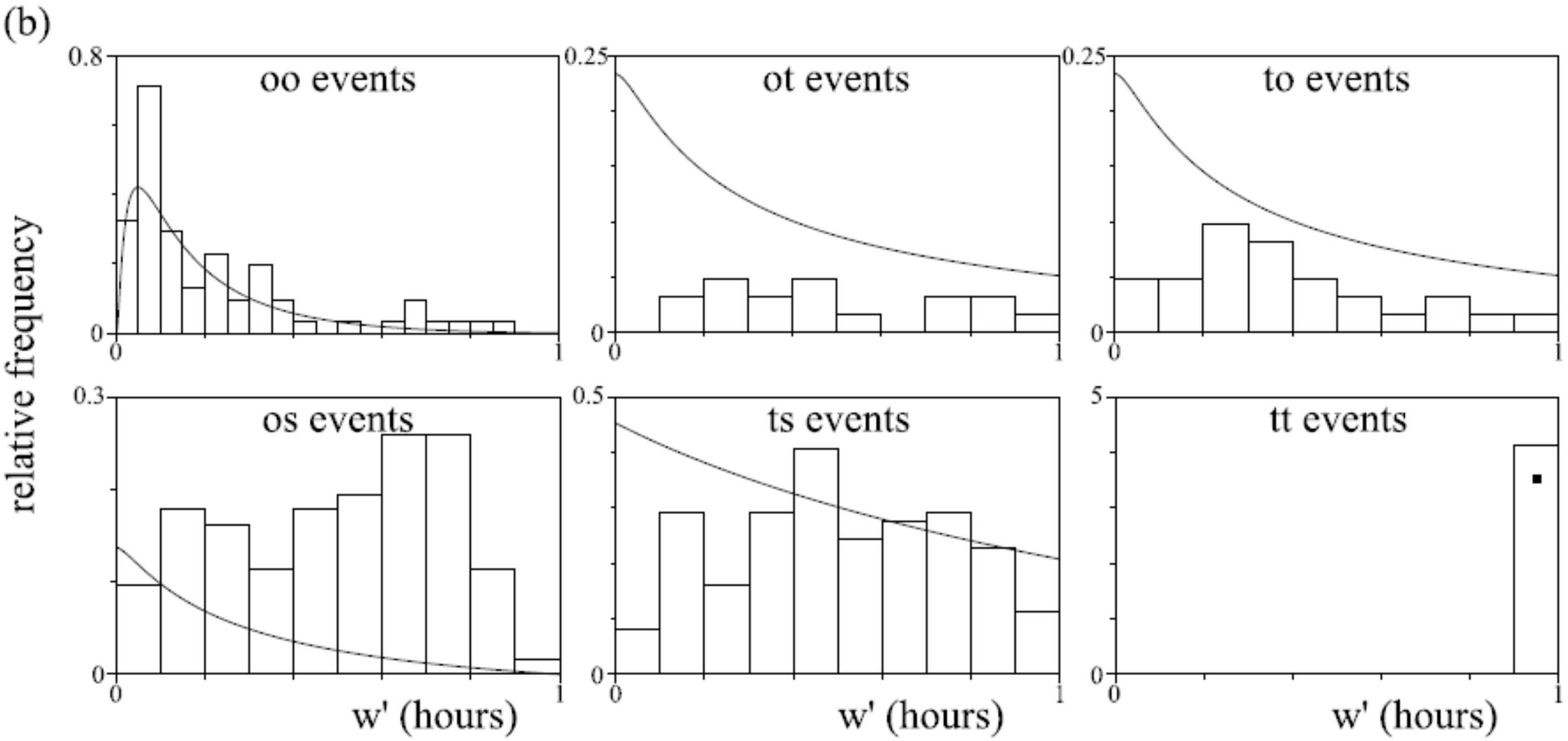




Assuming either a gamma distribution for g(x) (solid line), a lognormal (dotted), or the sum of two lognormals (dashed), we estimate very similar average contact times

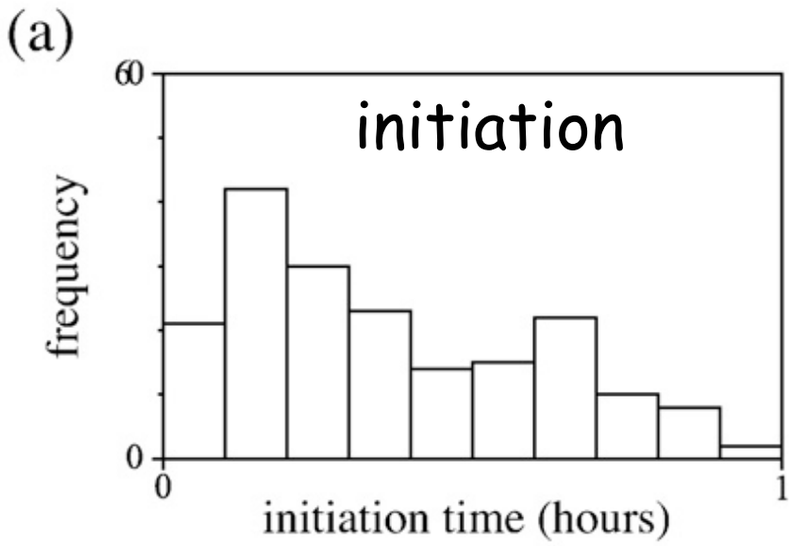
Fits suggest an exponential relation between observed and true contact time

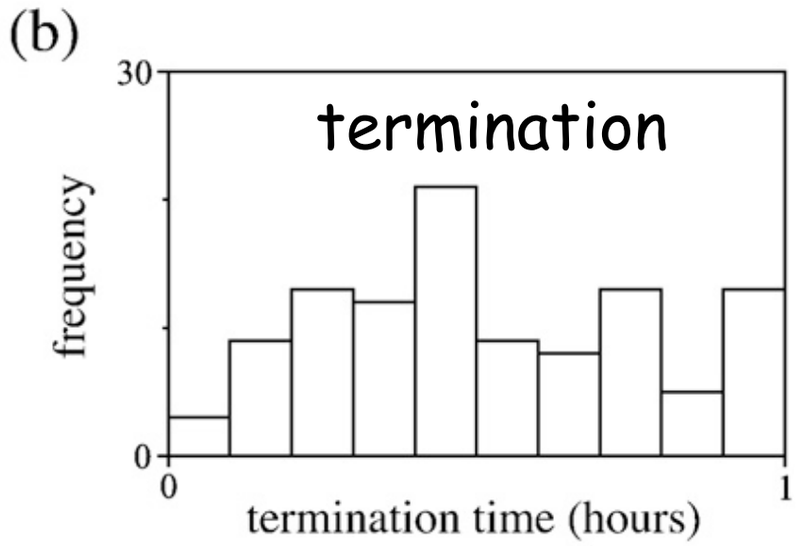
But fits are of poor quality

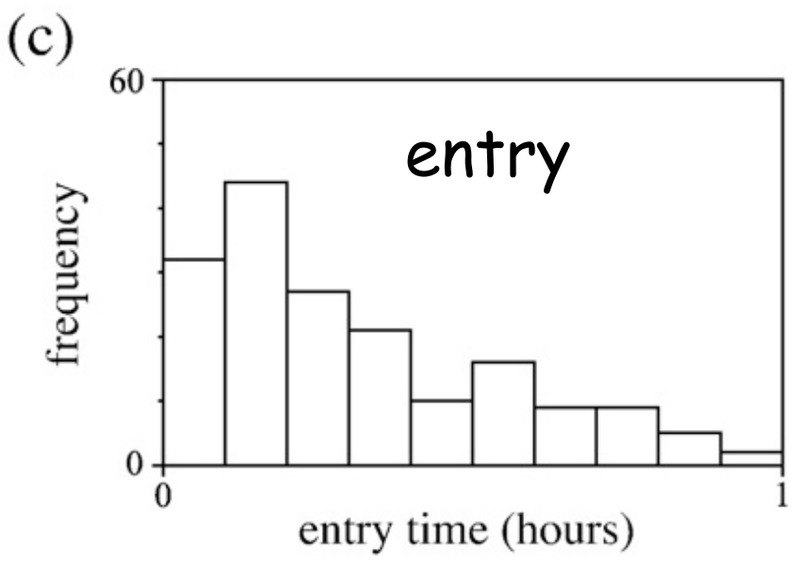


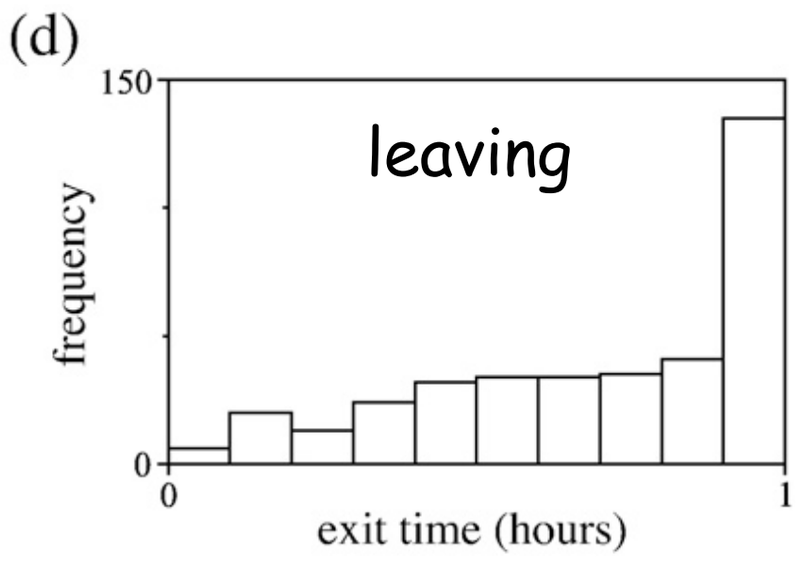
to, ot, os and ts events have to decline with the observed contact duration, but do not.

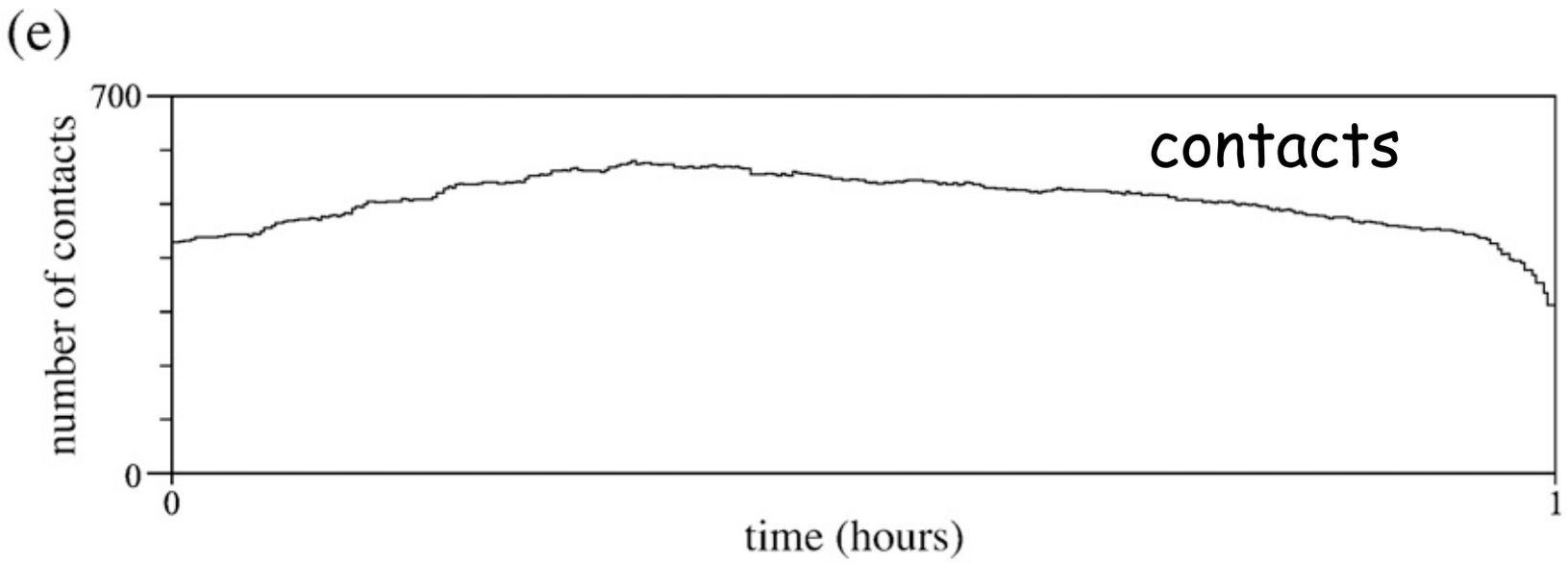
Check data for artifacts: tissue drift











Observed initiation (a), termination (b), entry (c) and leaving (d) events, and number of contacts should be constant over time.

Subset of 9/33 expts suggest x=5h

Beltman et al. Nature Revs Immunol 2009

Artefact or bias	How to detect	How to correct	
		S s	T (c
		D	C d
		E n	P f a
z		I z c	P a (f t s
		S	I i o a
		C p b	N
		T t c o	N

Conclusions

Parameter estimation is far from trivial

Observed contact times are biased by restricted time window and spatial area

True contact times can be estimated by fitting a complicated maximum likelihood model and/or by a simple shortcut method

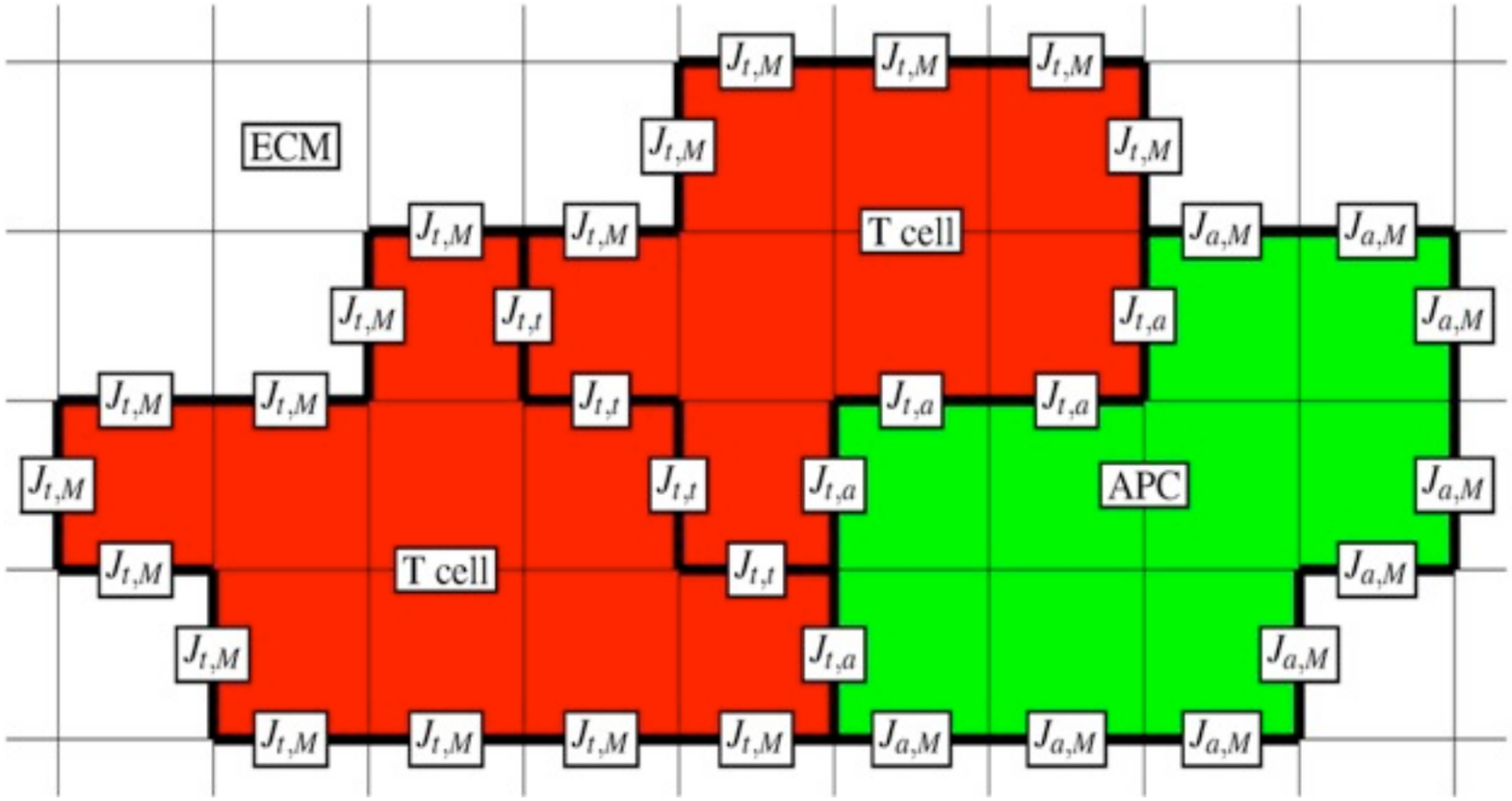
All data: 3.3h (2.8-3.8), Best data: 5h (1.7-7)

Beltman et al. J Immunol Methods 2009

Test data for artifacts like tissue drift

Beltman et al. Nature Revs Immunol 2009

Cellular Potts Model: grid



Cells have a target volume

Matrix of adhesion coefficients J between all cell types

asynchronous Cellular Automaton

Surface energies: Hamiltonian

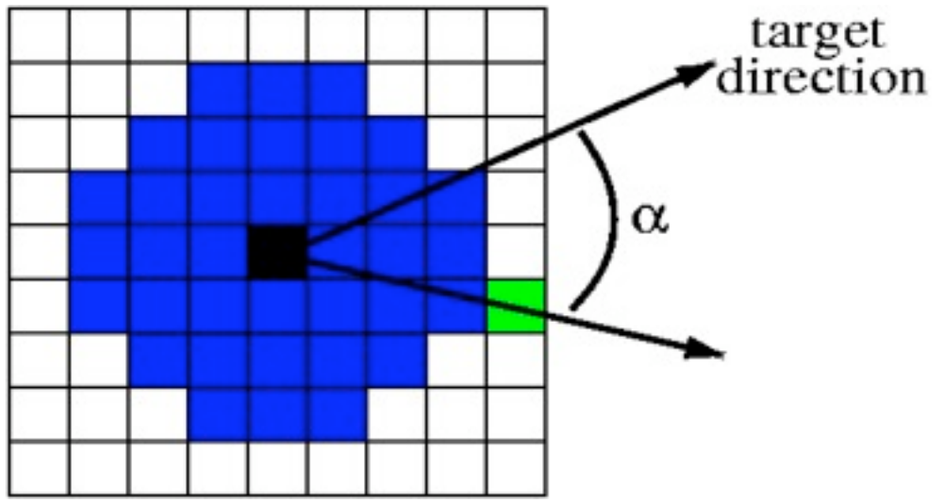
System minimizes its energy

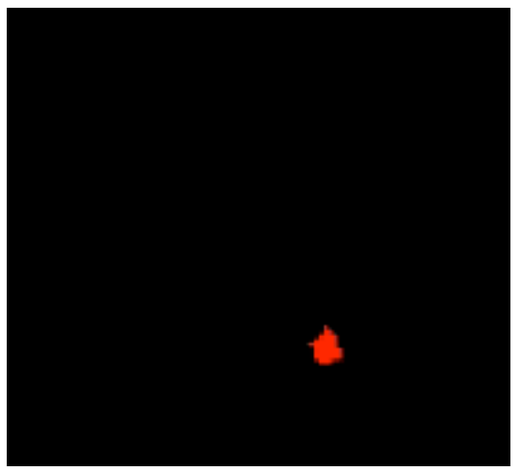
ΔH determines probability of copying

$$H = \Sigma J + \lambda (v - V_T)^2$$

T cells: target direction

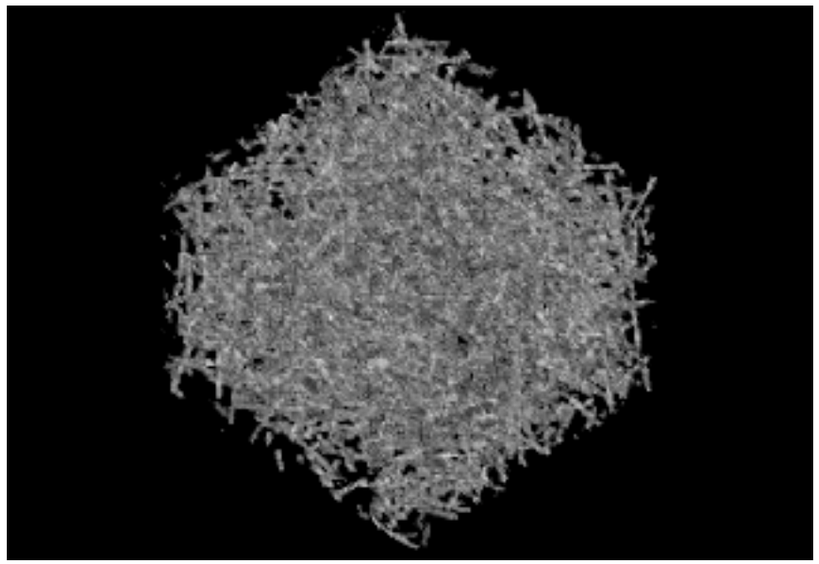
$$\Delta H = - \mu \cos(\alpha)$$





Adjust target direction according to recent displacement
(also directional persistence)

Model T cell area in LN: RT network



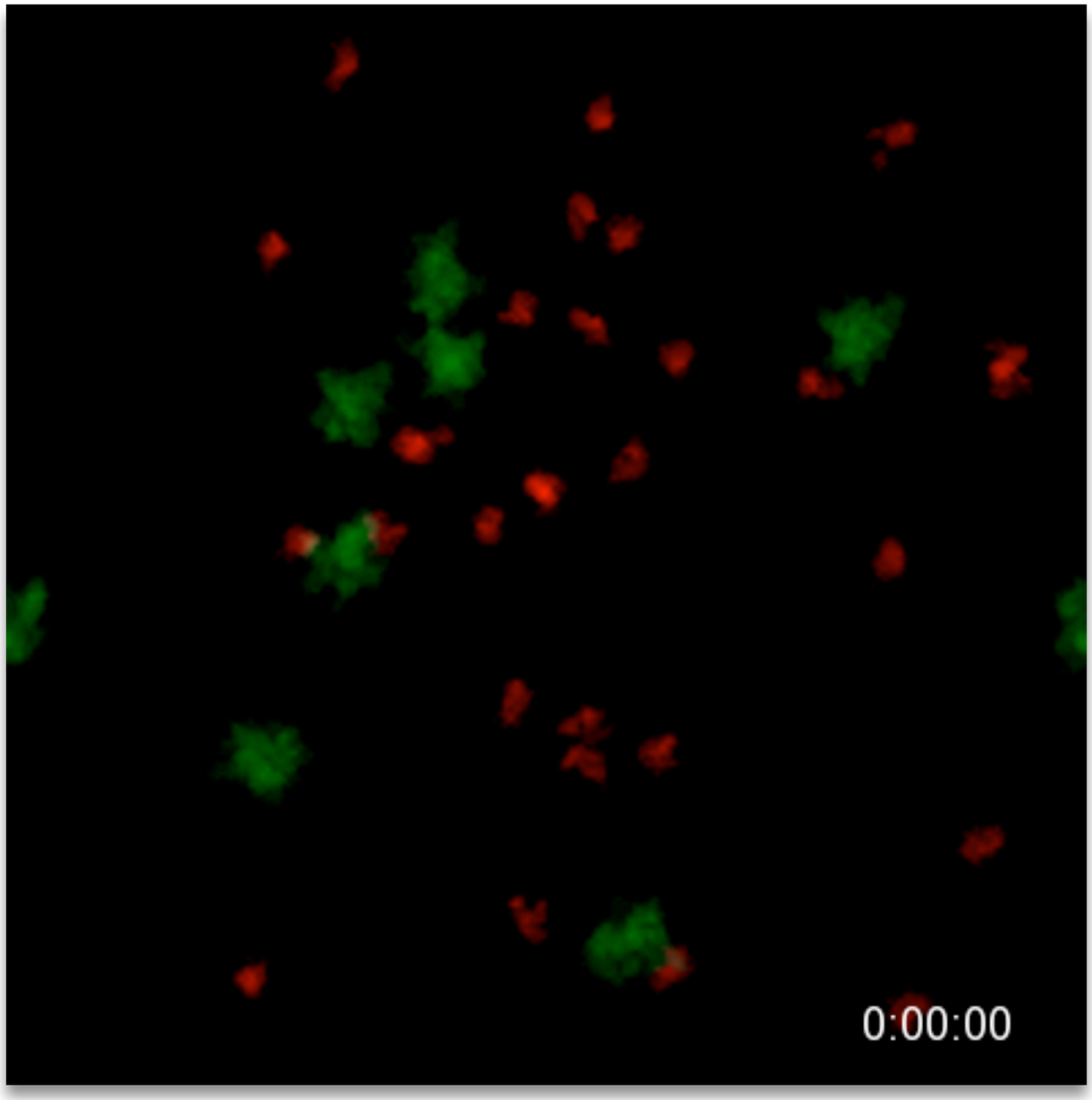
1 pixel = 1 μm^3

T cell: 150 μm^3, DC: 2200 μm^3

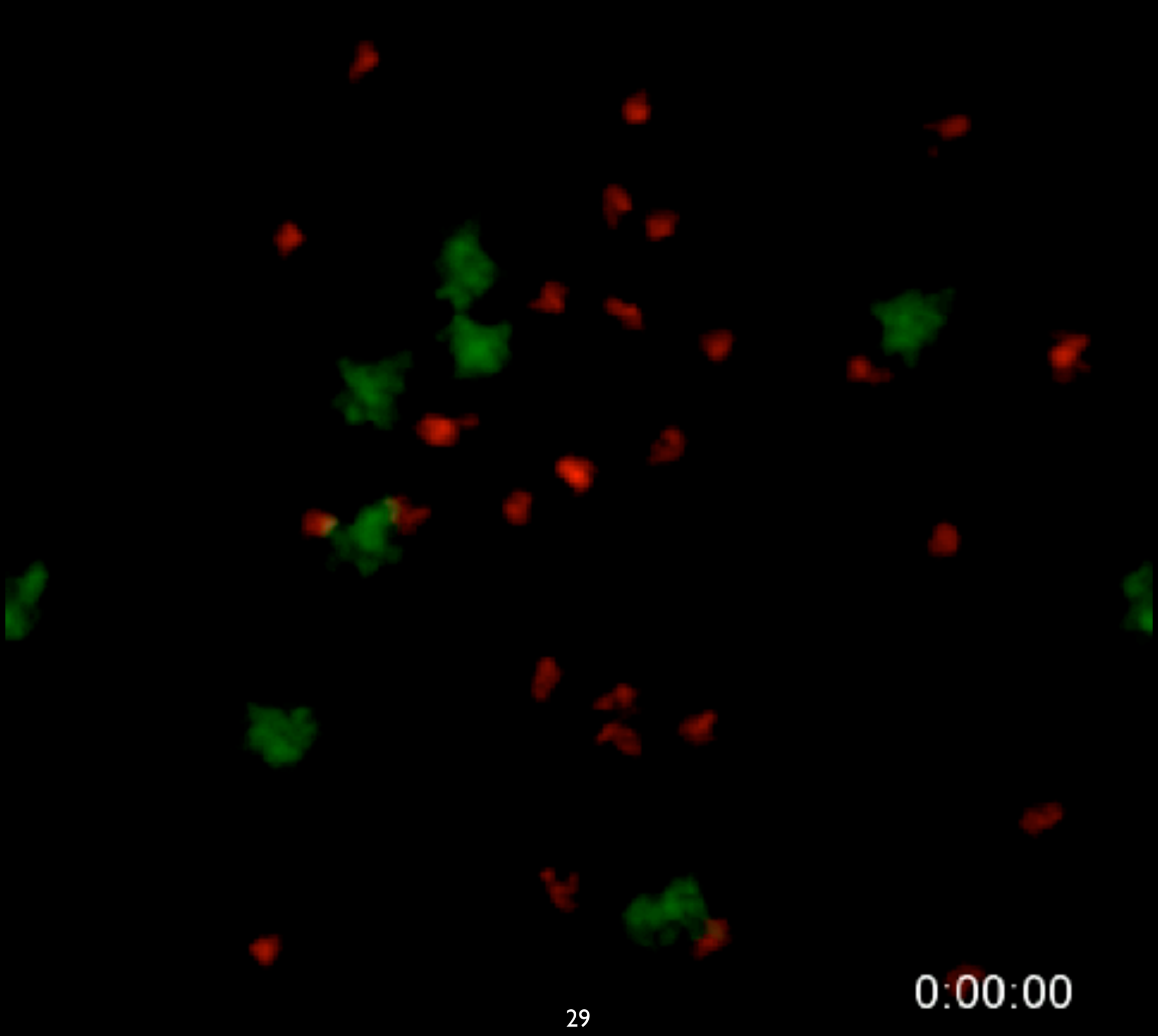
torus: 100 µm x 100 µm x 100 µm

static reticular network (rods)

Now with Antigen: red Ag specific T cells, green cognate DCs



During short contacts cells increase their adhesion for APCs, between contacts they slowly forget this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